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NUTRITION REVIEWS

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MAY 12 1952

NUTRITION REVIEWS

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ABUSE OF DATA ON THE BIOLOGIC VALUE OF PROTEINS

Much has been learned about the biologic value of proteins. This value depends upon their essential amino acid content and also possibly on the rate of release in the digestive tract of the essential amino acids. All this information has given us a fundamental understanding of protein nutrition.

Gelatin is recognized as an incomplete protein, and it so behaves biologically. Its freedom from an essential amino acid, such as tryptophan, accounts for this inferior behavior. The wheat kernel contains the alcohol-soluble protein gliadin, which is extremely low in an essential amino acid, lysine. Gliadin may constitute 40 per cent of the proteins of the wheat kernel. Forty per cent of the total protein of barley is hordein, also an alcohol-soluble protein. Fifty to 55 per cent of the proteins of the corn kernel is zein, an alcohol soluble-protein lacking in the amino acid, lysine. Rye also contains a gliadin, which constitutes 50 per cent of the total protein in rye. Whole oats, while not containing an alcohol-soluble protein, are nevertheless low in lysine. These facts help to explain in part why some of our cereal grains, such as barley, rye, corn, and wheat, all of which contain alcohol-soluble proteins low in lysine content, give inferior animal growth performance.

If these cereal grains constitute the sole article of diet, the protein alone, irrespective of other deficiencies, could not support normal growth of an animal. There are other deficiencies besides the relatively poor quality of the protein, but those deficiencies will not enter into this discussion.

It should be pointed out that these cereal grains do contain in the embryo (germ) proteins of high biologic quality. Investigators have shown that the proteins of the corn or wheat embryo are of a biologic value approximating those of dry skim milk, egg white, or ground beef. The biologic value of milk protein is often taken as the standard of highest efficiency. However, the germ of the seed constitutes a small proportion of its total weight, usually not more than 3 per cent, and thus the embryo proteins are diluted by the less efficient proteins of the endosperm.

For fifty years it has been known that the cereal grains alone would not support normal growth of domestic animals. Occasionally one would find a farmer feeding his young pigs such a combination as corn and middlings; corn, oats and an oil meal; or wheat and wheat bran, and wonder why they were not doing well. If this farmer were living in a dairy district and were also adding skim milk to any of the above combinations, good growth would follow.

Today it is well understood why there is this difference in growth between a corn-oat-oil meal ration and such a mixture supplemented with skim milk. One of the main effects, if not the most important, is the protein supplemental value of the skim milk. The essential amino acids lacking in quantity in the cereal mixture are increased by the skim milk addition.

The necessity of supplementing the cereal grain proteins with the better and more efficient proteins of milk, meat, or eggs has been known for more than forty years. Even the most efficient *proportions* of a mixture of cereal and animal proteins to obtain good growth have been known since 1918. In that year it was shown that the nitrogen retention by a growing animal (pig) was only 20 per cent of the total nitrogen ingested when the ration consisted of 600 g. of corn meal and 50 g. of skim milk. However, when the 600 g. of corn meal were supplemented with 600 g. of skim milk the nitrogen retention rose to 62 per cent of

the total nitrogen ingested, a difference of 40 per cent. A similar result would have been obtained with any other cereal grain.

For example, it took approximately the same proportions of rolled *oats* and skim milk to get a nitrogen retention equivalent to that found with corn and milk. In other words, the milk nitrogen must constitute about 25 to 30 per cent of the total nitrogen of the ration or diet in order to secure a maximum efficiency. One part by weight of a cereal plus one part by weight (volume is close enough) of milk will give an efficient protein mixture. This proportion applies to any cereal. In addition it makes for conservation and better use of the poor proteins of the cereal grains.

Such results established a principle of practical nutrition that is now forty years old, but too often forgotten by or unknown to the present generation. *Cereals and milk always go together*. This is the practical combination for efficiency for a growing child at breakfast time. True, muscle meat, glandular organs, or egg proteins are also excellent protein supplements to the cereal grain proteins; but in the United States they are less likely to be used in the case of young growing animals or children.

Recently there have come into the literature data showing differences in the nutritive value of the proteins of various breakfast cereals. As recognized long ago, the nutritive value of cereals, cooked or uncooked, is of a low order as compared with milk, eggs, or meat when fed as the sole source of protein. As a matter of fact, the early data postulated distinctly that any cereal breakfast food was to be served with milk, which dissipates any inferiority of the raw or processed cereal in respect to protein efficiency.

It is unfortunate that some have seized upon the results displayed in recent literature to claim a nutritional superiority of one cereal over certain others. This practice is unwarranted, unethical, and not in the interest of the public good. Breakfast cereals are eaten with milk, only rarely with water alone. Any differences in the biologic value of their proteins is obliterated when consumed with the proper proportions of milk—a weight ratio of 1:1.

The time is past when we relegate a food to the inferior class because its proteins, *when fed alone*, are not of high biologic value. What we have learned and should continue to stress is the importance of knowing how to supplement the poor protein food with those common available foods that will raise the efficiency of the mixture in respect to the protein. Corn should not be condemned because its proteins, *when fed alone*, are not so efficient as those of the oat kernel. Mississippi Valley farmers should not and would not abandon the raising of corn because someone informed them that the proteins of their product were inferior to the proteins of another cereal. The question of the comparative biologic value of the proteins of cereal breakfast foods is academic insofar as one can be sure that they are accompanied regularly by a generous intake of milk, as represented by common practice and sound nutritional teaching.

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METABOLISM OF WATER AND ELECTROLYTES IN PATIENTS WITH LIVER DISEASE

When the intake of sodium in food and from other sources is strictly limited, ascites and edema formation ceases in patients with liver disease subject to this complication (*Nutrition Reviews* **7**, *345 (1949)*). Although this dietary restriction in sodium has come to be an important therapeutic measure, the reasons for its effectiveness and, for that matter, the explanation of ascites and edema formation in chronic liver disease have not been clarified.

Work reported by W. E. Ricketts, L.

Eichelberger, and J. B. Kirsner (*J. Clin. Invest.* **30,** *1157 (1951)*) confirms and enlarges upon studies previously reviewed. The failure of patients with cirrhosis of the liver who are forming ascites to excrete sodium in the urine irrespective of sodium intake was observed again. The data these authors present suggested to them that the primary disturbance resulting in ascites and edema accumulation was the retention of sodium. The studies reported by these authors are based on observations of 7 patients with cirrhosis of the liver, 4 of whom had ascites and edema at the time of study. In addition, 2 normal individuals served as controls. A constant daily intake of calories, water, and electrolytes was provided during the study period.

In general, the intravenous administration of normal human serum albumin did not increase the excretion of sodium except in one patient. Demonstrable salt depletion, decrease in blood sodium, or diminution of urine output were not observed in these patients maintained on restricted sodium intake. The failure to observe diminished urine output in these patients suggested to the authors that an antidiuretic principle found in the urine, as, for example, by E. P. Ralli, J. S. Robson, D. Clarke, and C. L. Hoagland (*J. Clin. Invest.* **24,** *316 (1945)*), was not acting primarily on water retention but rather on sodium retention. Ralli and co-workers suggested that the posterior antidiuretic hormone was present in large quantities in the urine of patients with cirrhosis of the liver and with ascites and edema, because the liver failed to inactivate this hormone.

A. G. White, G. Rubin, and L. Leiter (*J. Clin. Invest.* **30,** *1287 (1951)*) concluded that both endogenous and administered antidiuretic hormones (pitressin) were inactivated at approximately the same rate in patients with cirrhosis of the liver as in normal individuals. Nine patients with cirrhosis of the liver and 8 control subjects were studied in this investigation. The rapid intravenous injection of 0.57 micrograms per kilogram of body weight in subjects continuously hydrated by intravenous 5 per cent glucose in water was the same in the patients with liver disease as it was in the controls. The fact that the time required for maximal diuresis to occur with the glucose infusion prior to the intravenous administration of pitressin was approximately the same for the two groups of patients (normal and cirrhotics) suggested to the authors that not only was exogenous antidiuretic hormone metabolized normally, but that the inactivation of the endogenous hormone likewise proceeded at an approximately normal rate. However, delayed water diuresis has been observed in the past in patients with cirrhosis and the nocturia so often found has been attributed to this. The explanation usually given has been failure of the liver to inactivate pituitary antidiuretic hormones.

An explanation for the nocturnal diuresis of water and sodium in cirrhosis of the liver and also in congestive heart failure is the subject of a study reported by R. Goldman (*J. Clin. Invest.* **30,** *1191 (1951)*). In this study 8 patients without hepatic or renal disease served as controls. Constant control of food intake was maintained. Eight patients with heart disease, and 13 with cirrhosis of the liver either accompanied by ascites, or with a history of ascites in the recent past, were studied. Clear-cut confirmation of nocturnal diuresis was observed in 6 of the patients with congestive heart failure and in 8 of the 13 with cirrhosis of the liver. Sodium, potassium, and creatinine excretions were also measured. In general, low sodium and potassium excretions followed those of water in the control subjects, whereas creatinine was constant throughout the study. In the patients with congestive heart failure and hepatic cirrhosis much greater variations in sodium, potassium, and creatinine excretion were observed; however, sodium diuresis did occur at night both in the patients with heart failure and in those with cirrhosis of the liver. A consistent relationship between creatinine and sodium

excretions was not found, so that it was impossible to prove an increased nocturnal filtration rate. The authors were unable to conclude whether a humoral agent elaborated or inactivated normally by the liver, or some abnormality in cardiac output and glomerular filtration accounted for the nocturnal diuresis in these two conditions.

The problem of renal function in patients with cirrhosis of the liver has certainly not been settled. A recent study reported by S. H. Leslie, B. Johnston, and E. P. Ralli (*J. Clin. Invest.* **30,** *1200 (1951)*) indicated that effective renal plasma flow, glomerular filtration rate, and the tubular absorption of para-aminohippurate were reduced when ascites occurred in a group of patients with cirrhosis of the liver. When treatment in these cases relieved the need for further paracenteses, and therefore presumably prevented fluid retention, renal function returned to normal or was even somewhat high. Similar observations made by A. J. Patek *et al.* (*J. Clin. Invest.* **27,** *135 (1948)*) did not reveal abnormalities in renal function in 3 patients with cirrhosis of the liver studied at that time, both before and after the intravenous administration of concentrated human serum albumin.

Thus, from a nutritional standpoint, it is possible to treat ascites and edema accumulation in patients with cirrhosis of the liver effectively with a low sodium diet. Moreover, these patients seem to tolerate a low sodium diet better than patients with heart failure or particularly those with hypertension. Unquestionably, the high portal pressure and low serum colloid osmotic pressure play a part, if not a primary role, in the genesis of ascites in these patients. The explanation for the delayed water diuresis in some patients, nocturnal diuresis in many others, and failure to excrete administered sodium in almost all, must await further explanation. The studies reviewed here do not indicate that alterations in renal function or an inactivation of antidiuretic hormone (endogenous or exogenous) entirely account for this distressing complication in cirrhosis of the liver.

HEXOSE PHOSPHATE OXIDATION AND PENTOSE FORMATION

The course of glucose oxidation in animal tissues is generally described as phosphorylation followed by conversion to fructose phosphates, scission to triosephosphates, and eventual formation of lactic acid. This process of *glycolysis* has been extensively investigated, and the many soluble enzymes involved have been extensively purified or crystallized. Formation of lactic acid from glucose-6-phosphate can take place anaerobically, while lactic acid formation in tissues is decreased in the presence of oxygen. This is the well-known Pasteur effect, but the mechanism of the phenomenon is still discussed (*see* D. Burk, *Cold Spring Harbor Symposia on Quantitative Biology* **7,** *420 (1939)*). One attractive suggestion for the mechanism is that glucose-6-phosphate can be oxidized directly through triphosphopyridine nucleotide (TPN), flavoprotein, and the cytochrome system.

The suggestion that such a mechanism is possible stems from the work of O. Warburg, W. Christian, and A. Griese (*Biochem. Z.* **282,** *157 (1935)*), and F. Dickens (*Biochem. J.* **32,** *1626 (1938)*). The former workers have described a dehydrogenase causing the oxidation of glucose-6-phosphate to phosphogluconic acid, and an enzyme complex which further converted the gluconic acid derivative to phosphoketogluconic acid and other unidentified products. Dickens also studied the degradative oxidation of phosphogluconic acid and concluded that pentoses were included among the products. These early studies have a dual interest, namely, the mechanism of glucose oxidation and the biologic formation of pentoses. The physi-

ologic significance of pentose formation from decarboxylation of gluconic acid suffers from the fact that the product of such a reaction would appear from stereochemical considerations to be arabinose, rather than the ribose found in nucleotides and nucleic acids.

Only in the past few years has there been a renewed interest in the direct oxidation of phosphogluconic acid and pentose formation. B. L. Horecker (*Fed. Proc.* **9,** *185 (1950)*) reported a purified enzyme from yeast which quantitatively converts phosphogluconic acid to pentose phosphate and carbon dioxide. The enzyme also occurs in liver, kidney, brain, heart muscle, and red cells, indicating the broad significance of the reaction in metabolism. The pentose phosphate formed was hydrolyzed by phosphatase at a much slower rate than ribose phosphate, and was therefore not identical with the latter.

At about the same time, S. S. Cohen and D. B. M. Scott (*Science* **111,** *543 (1950)*) also reported the formation of pentose phosphate from phosphogluconic acid by yeast enzymes. These workers employed paper chromatography to study and identify the sugar phosphates present in the reaction mixture. A variety of hexose and pentose phosphates were first studied to aid in the identification of the unknown products of the enzyme reaction. Among the sugar phosphates formed was one with the R_f chromatographic characteristics of ribose phosphate. Furthermore, after alkaline phosphatase hydrolysis, the mixture of neutral sugars formed contained ribose as determined by fermentation with an adapted *Escherichia coli* strain. The results appear to establish that the naturally occurring ribose is a product of phosphogluconic acid oxidation, and that an inversion process must occur during the enzymatic reactions. It is of interest that glyceraldehyde-3-phosphate was also indicated by the chromatogram, from which it might be deduced that phosphogluconic acid oxidation might join the classic glucose metabolism scheme at the triosephosphate stage.

Further work by B. L. Horecker and P. Z. Smyrniotis (*Arch. Biochem.* **29,** *232 (1950)*) proved the formation of ribose-5-phosphate from phosphogluconate by a relatively purified yeast enzyme preparation. This enzyme preparation (phosphogluconic acid dehydrogenase) has been described by Horecker and Smyrniotis (*J. Biol. Chem.* **193,** *371 (1951)*). In order to accomplish complete oxidation of the phosphogluconate to pentose phosphate, pyruvate and lactic dehydrogenase were employed to cause reoxidation of reduced triphosphopyridine nucleotide.

The exact nature of the pentose phosphates formed in phosphogluconate oxidation was reported by B. L. Horecker, P. Z. Smyrniotis, and J. E. Seegmiller (*J. Biol. Chem.* **193,** *383 (1951)*). It was found that the proportion of the two pentose phosphates depended on the time of incubation of the enzyme and substrate. The first product of the reaction was identified as ribulose-5-phosphate, a ketopentose. The second was ribose-5-phosphate, with no evidence of arabinose phosphate. The pentoses were identified by chromatographic procedures coupled with chemical properties and hydrazone derivatives. In order to explain the results, the authors have postulated that oxidation of phosphogluconate occurs in the number 3 position to give a 3-ketophosphogluconate. Upon decarboxylation the latter would yield the identified product, ribulose-5-phosphate. A pentose phosphate isomerase produces ribose-5-phosphate from the ketopentose.

These studies represent a real advance in the knowledge of the metabolism of glucose, and it seems probable that further pursuit of pentose phosphate oxidation will bring this pathway of carbohydrate metabolism into the already known schema of metabolism. Since these studies also show that ribose phosphate may be formed from

glucose, this mechanism may represent the physiologic mechanism of pentose formation, since ribose (and desoxyribose) are the essential pentoses in nucleotides and nucleic acid. Pentose formation by condensation of triosephosphate with glycolaldehyde by the enzyme aldolase has been reported (E. Racker, *Fed. Proc.* **7,** *180 (1948)*), but there is more direct evidence that phosphogluconate oxidation is a source of the pentose in nucleic acids. Thus M. R. Stetten and DeW. Stetten (*J. Biol. Chem.* **187,** *241 (1950)*) have administered isotopically labeled gluconic acid to rats, and have isolated the liver nucleic acid which was rich in isotope. The specific activity of the nucleic acid pentose was not determined, but the finding supports the thesis that gluconate may serve as the precursor of pentose in nucleic acid.

DESTRUCTION OF THIAMINE IN PURIFIED DIETS

In 1949 L. V. Hankes and C. A. Elvehjem (*Proc. Soc. Exp. Biol. Med.* **72,** *349 (1949)*) described a disease in rats which they considered to be a separate and distinct deficiency syndrome. The condition was characterized by an arched back, oscillation of the head, loss of equilibrium, and convulsions followed by death. These signs developed in young animals after ten to twelve weeks on a purified, low protein diet. The ration (supplemented with DL-phenylalanine and DL-methionine) was niacin deficient, but contained all other known vitamins, including 0.2 mg. per cent thiamine hydrochloride. The development of the deficiency syndrome was delayed when the casein of the diet was increased to 18 per cent, and it was completely prevented by supplements of niacin or tryptophan.

Newer studies have shown that this syndrome is caused by lack of vitamin B_1 (R. L. Lyman and C. A. Elvehjem, *Proc. Soc. Exp. Biol. Med.* **77,** *813 (1951)*). Eighty micrograms of thiamine given intraperitoneally alleviated the convulsions and cured all other symptoms; continued injections restored normal growth and appearance. Furthermore, it was observed that 1.5 per cent succinylsulfathiazole in the diet afforded protection.

The fact that thiamine deficiency can be produced by a purified diet to which 0.2 mg. per cent of this vitamin has been added deserves special attention. The results might serve to show that there are pitfalls in nutritional experimentation of which one must be continually conscious even when using long established procedures. Supplementation of purified regimens with 0.2 mg. per cent (or even less) of thiamine hydrochloride is a standard method in many laboratories doing nutrition research. Lyman and Elvehjem reported several circumstances which were contributing to the development of the unexpected thiamine deficiency. It was found that part of the vitamin was destroyed in the diet. In addition, the dietary intake under their experimental conditions was very low. Chemical analysis of the rations disclosed that about 50 per cent of the vitamin was destroyed in a matter of a few hours and 70 to 75 per cent was lost after six days. The exact mechanism of the destruction of thiamine in the diet was not determined; however, the authors discussed the possibility that the destruction might have been brought about by components of the synthetic salt mixture. This mixture contained, among other minerals, potassium iodide, copper sulfate and ferric citrate, compounds which are widely used as ingredients in purified rations, and which are well known to be capable of participating in oxidation and reduction reactions. Ferrous iron has been found to destroy thiamine under certain conditions (E. De Ritter and S. H. Rubin, *Ind. Eng. Chem. (Anal. Ed.)* **19,** *243 (1947)*). It should be recalled that there are several other well-known thiamine-destroying mechanisms (*Nutrition Reviews* **4,** *222*

(*1946*)). The vitamin is inactivated by heat, and it is disintegrated by bisulfite and by the enzyme thiaminase, present especially in raw fish. Thiamine has also been reported to be inactivated by an unidentified nonenzymatic principle present in rice polishings, wheat germ, and various seeds (K. Bhagvat and P. Devi, *Indian J. Med. Res.* **32,** *131* (*1944*)).

The finding that succinylsulfathiazole prevented development of the deficiency was explainable since chemical assays demonstrated that thiamine was quite stable when 1.5 per cent of this sulfa drug was added to the diet. The mechanism of the protective action of the sulfa drug against destruction of thiamine in such a synthetic diet is not known.

The other point in consideration is that the low protein, niacin-free diet induces a serious decrease in food consumption, as most other deficient diets do. Lack of appetite thus aggravates the situation; it inflicts a further decrease in thiamine intake—and also in the intake of other dietary factors. When 18 per cent casein, or niacin-tryptophan was added to the low protein diets from the beginning of the experiment, food consumption was greatly increased, and this in turn automatically induced an increased intake of thiamine. The prevention of the vitamin B_1 deficiency syndrome by niacin-tryptophan administration is very likely to be caused by this mechanism. It seems possible that this explanation applies equally well to other phenomena observed in animals and in humans on low protein diets.

AMINO ACID IMBALANCE

The growth depression in rats which can be demonstrated from the addition of certain amino acids to presumably adequate and balanced diets has received considerable attention in the past few years. Interest in this problem grew out of the observation by workers at the University of Wisconsin that severe growth depression resulted when dietary casein was partially replaced by the proteins of corn (W. A. Krehl, L. M. Henderson, J. de la Huerga, and C. A. Elvehjem, *J. Biol. Chem.* **166,** *531* (*1946*)). This particular situation was caused by a deficiency of tryptophan and could be corrected by supplying niacin. The same condition was also produced by feeding wheat, gluten, zein, gelatin, or acid-hydrolyzed casein, all of which are deficient in tryptophan.

Mixtures containing casein and gelatin produced an imbalance that could not be corrected by substituting dextrin, glucose, or cornstarch for the sucrose in such diets. On the other hand, these carbohydrates were effective in correcting the imbalance resulting from corn and wheat gluten diets.

These observations indicated that amino acids other than tryptophan were probably involved (L. M. Henderson, T. Deodhar, W. A. Krehl, and C. A. Elvehjem, *J. Biol. Chem.* **170,** *261* (*1947*); L. V. Hankes, Henderson, W. L. Brickson, and Elvehjem, *Ibid.* **174,** *873* (*1948*)). It was further noted that glycine, DL-phenylalanine and DL-threonine depressed growth on a niacin-deficient casein-sucrose diet, but not on a similar diet containing dextrin as the carbohydrate source. Later these workers noted that these amino acids depressed growth on the casein-sucrose diet only when such diets contained supplementary cystine or methionine (Hankes, Henderson, and Elvehjem, *Ibid.* **180,** *1027* (*1949*)).

In a recent paper from the Wisconsin laboratory (R. L. Lyman and C. A. Elvehjem, *J. Nutrition* **45,** *101* (*1951*)), further studies are reported on the amino acid imbalances that result from the addition of various amino acids to a casein-gelatin diet. Weanling male rats were fed the various diets for a five-week experimental period. Growth depression or gross evidence of niacin deficiency (porphyrin exudate around

the nose and eyes and general emaciation) served as a criterion in determining the effect of the diet alterations.

The basal diet had the following percentage composition: dextrin (or sucrose), 81.8; corn oil, 5; casein, 9; salts, 4; L-cystine, 0.2. Vitamin additions consisted of the following (milligrams per 100 g. of diet): thiamine, 0.2; riboflavin, 0.3; pyridoxine, 0.25; calcium pantothenate, 2; choline chloride, 100; inositol, 10; biotin, 0.01 and folic acid, 0.02. Fat-soluble vitamins were fed once weekly at levels to supply the following daily average intake per rat: vitamins A and D, 400 and 4 I.U. respectively, and vitamins E and K, 0.7 and 0.06 mg. respectively.

The addition of 6 per cent of gelatin to the dextrin-basal diet at the expense of an equal amount of carbohydrate component resulted in severe growth depression due to tryptophan deficiency. Rats receiving this diet made weight gains equal to only 40 per cent of the gains obtained on the basal diet. Omission of the added cystine from the diet resulted in gains equal to 80 per cent of that obtained on the basal diet. Although growth was less rapid, similar results were obtained in the presence and absence of cystine on the sucrose-basal diet. These results indicated that the tryptophan deficiency induced by gelatin was much more severe in the presence of dietary cystine than in its absence.

Acid-hydrolyzed gelatin produced results similar to those obtained with plain gelatin, and certain amino acid mixtures likewise produced the same severe growth depression. Mixtures tested were: (1) an amino acid mixture simulating the amino acids present in 6 per cent of dietary gelatin; and (2) 3 amino acid mixtures, each of which contained five amino acids as they occur in 6 per cent of dietary gelatin. These latter 3 mixtures contained the following:

Mixture 1	*Mixture 2*	*Mixture 3*
arginine	histidine	aspartic acid
phenylalanine	serine	alanine
threonine	leucine	lysine
glycine	valine	tyrosine
proline	glutamic acid	isoleucine

The amino acid mixture simulating gelatin behaved like gelatin, producing a severe growth depression on the basal diet and a lesser depression on the basal diet devoid of the cystine supplement.

Amino acid mixture 1 produced severe growth depression only when it was further supplemented with alanine or when this mixture and mixture 3 were fed together. The combination of mixtures 1 and 2 produced somewhat less growth depression than the combination of mixtures 1 and 3.

These results emphasized that alanine appeared to be the important amino acid in precipitating the amino acid imbalance with gelatin. However, the rather marked depression (approximately 60 per cent of the growth in rats receiving the basal diet) noted with mixtures 1 and 2 suggested that sheer numbers of amino acids were effective in initiating the imbalance.

A series of studies was also conducted to see if the niacin-tryptophan deficiency could be produced if some of the amino acids in mixture 1 were omitted. Since all of the various combinations tested were ineffective, it would seem that arginine, phenylalanine, threonine, glycine, proline, and alanine were all involved in producing the imbalance.

These results serve to emphasize the delicate balance that exists between the amino acids and the ease with which drastic deficiency states can be induced by disturbing the balance. These studies further emphasize that 9 per cent casein diets lend themselves to drastic tryptophan imbalance studies only if the sulfur-amino acids (cystine or methionine) are supplied as supplements. The difference in behavior of sucrose diets and dextrin diets appears to be that the latter permit better growth and that such growth precipitates a deficiency of sulfur-amino acids.

Thus far the amino acids that have received the most attention in imbalance studies are those that are related to other nutrients in function. Tryptophan imbalance is aggravated by a deficiency of niacin, and

methionine imbalance is aggravated by a deficiency of choline.

In the tryptophan deficiency imbalance, niacin appears to be much more effective as a corrective agent than tryptophan itself, an implication that such imbalances actually result in a decrease in efficiency of amino acid utilization.

Whether or not this is a generalized effect must await studies of imbalance situations where amino acids not directly related in their function to other nutrients are limited.

GALACTOSE UTILIZATION AND CONVERSION TO GLYCOGEN

The dependence of the young mammal upon lactose for a considerable part of its food energy for some time after birth and the presence of lactase in the intestinal juice have focused attention upon the value as well as the limitations of galactose from the point of view of nutritional biochemistry. Once milk sugar is hydrolyzed in the intestine, the constituent galactose is readily absorbed; indeed, its rate of absorption exceeds that of glucose, fructose, mannose, xylose, and arabinose as measured in laboratory rats (C. F. Cori, *J. Biol. Chem.* **66,** *691* (*1925*)).

When the heat production and respiratory quotient were measured in man following the ingestion of the hexoses, galactose showed a response slower than fructose but faster than glucose, but there seemed to be no correlation between the speed of response of the respiratory quotient and the level of blood sugar (H. J. Deuel, *J. Biol. Chem.* **75,** *367* (*1927*)). The tolerance for galactose given by mouth is much greater than that for glucose in the human subject, and it appears that the utilization of galactose by the diabetic differs little from that in the normal person (J. H. Roe and A. S. Schwartzman, *Ibid.* **96,** *717* (*1932*)). After a four-day fast, the decrease in acetone excretion of human subjects was greater after galactose administration than with glucose (Deuel, M. Gulick, and J. S. Butts, *Ibid.* **98,** *333* (*1932*)).

The rat appears to form glycogen from galactose more slowly than from glucose (C. F. Cori, *Proc. Soc. Exp. Biol. Med.* **23,** *459* (*1926*)), but if galactose is given for seven to twelve days and the rat is then fasted seventy-two hours, the glycogen of the liver seems to be retained longer than the glycogen from other sugars (H. J. Deuel *et al.*, *J. Biol. Chem.* **101,** *301* (*1933*)). This conclusion appears to be supported by the observation that the number of glucose units in hepatic glycogen of galactose-fed rabbits is larger than in glycogen synthesized in these animals after feeding glucose (D. J. Bell, *Biochem. J.* **30,** *1612* (*1936*)). However, more recent studies, in which the liver glycogens formed after administration of various sugars were compared on the basis of end group assay values, have failed to support the view that the glycogen formed from glucose differs from that formed from galactose in the rabbit (M. Schlamowitz, *J. Biol. Chem.* **188,** *145* (*1951*)), or in the rat (M. Morrison, A. C. Kuyper, and J. M. Orten, *Fed. Proc.* **10,** *226* (*1951*)).

It is well known that modest amounts of absorbed galactose are converted to glucose in the organism. These two hexoses differ from each other only in the steric configuration about the fourth carbon atom; a recent study has thrown light upon the nature of the change from one sugar to the other (Y. J. Topper, and DeW. Stetten, *J. Biol. Chem.* **193,** *149* (*1951*)). Three pathways are suggested: (1) epimerization may take place about C-4 as the sole change; (2) one hexose may be formed from fragments resulting from the degradation of the other; (3) the hexose molecule may undergo rearrangement to a cyclohexitol intermediate from which the other hexose is, in turn, produced. To determine which of these possibilities is effective, rats were given galactose-1-C^{14} (0.5 g. sugar in 3 ml. water) by stomach

tube. After three hours glycogen was isolated from the livers and the distribution of C^{14} in the molecule of the constituent glucose was determined after hydrolysis. The labeled galactose contained 78,600 counts per minute per milliatom of carbon, and it was found that 11 per cent of the C^{14} administered was excreted in the urine. From the comparative counts of the galactose-1-C^{14} administered and of the liver glycogen, it was found that over 50 per cent of the glycogen had its origin in the galactose. Carbons 1 and 2 of the glucose resulting from the hydrolysis of the hepatic glycogen were identified through two successive Wohl degradations; periodate oxidation of the glucose as well as of the glucosazone defined the C-6 (C-4 + C-5 and C-1 + C-2 + C-3). By this means the localization of the radioactivity was made with respect to the individual carbon atoms in the glucose formed from labeled galactose by way of glycogen. The radioactive carbon was found almost exclusively in the C-1 of the glucose, the position corresponding to that in the administered galactose. From these observations it may be concluded that the chief pathway whereby galactose is converted to glucose in the body of the rat is an epimerization at the C-4 of the galactose, inasmuch as the radioactivity was not shifted to other carbons in the glucose as would have occurred had the other suggested pathways been utilized.

The precise mechanism of this epimerization is still to be revealed. However, the recent discovery (R. L. Garner and G. F. Grannis, *Science* **114,** *501 (1951)*) of phosphogalactoisomerase, an enzyme in *S. marxianis* which catalyzes the conversion of galactose-1-phosphate to glucose-1-phosphate, may point the way to a means of further elucidation of this problem.

OBESITY

Of the many forms of malnutrition which occur in the United States, obesity is unquestionably the most common in occurrence and the most serious in terms of the total years of life sacrificed. Yet oddly enough, it is one of the very few diseases, of the many to which man is susceptible, which can invariably be cured, provided the therapeutic regimen is rigidly followed. Unfortunately, these facts are known to too few professional as well as nonprofessional people. Research and interest in undernutrition has always been much greater than in overnutrition. Indeed, by many the term malnutrition is even used synonymously with undernutrition. It is therefore pleasing to observe that D. B. Armstrong, L. I. Dublin, G. M. Wheatley, and H. B. Marks (*J. Am. Med. Assn.* **147,** *1007 (1951)*) have clearly defined and summarized the ravages of obesity in modern times. The data were obtained from the Statistical Bureau and the Health and Welfare Division of the Metropolitan Life Insurance Company.

Defining overweight as any deviation of 10 per cent or more above the ideal weight for the person, the authors estimate that at least one-fifth of the population of the United States over the age of 30 is overweight. This represents about 15 million people. Considering that a weight of 20 per cent or more above the ideal constitutes pathologic overweight or obesity which definitely requires correction, it is estimated that the number of obese adults is 5 million or more. Considered in terms of the number of patients annually in the United States with beriberi, scurvy or pellagra, this figure is astronomic.

The several studies which have been made in the past by life insurance companies on mortality in relation to body build have shown consistently that among overweight persons there is a substantially higher mortality than among persons of ideal weight.

The results of the newer study, based on 25,998 overweight men and 24,901 overweight women, are even more startling.

The overweight men studied had a mortality in the aggregate one and one-half times that of insured men of ideal weight. When the overweight men were divided into groups according to the age at examination for insurance, the mortality, relative to normal, was greatest in the younger ages and least at older ages. Thus, in the groups aged 20 to 29 at examination the number of subsequent observed deaths was 80 per cent greater than expected by standard experience as compared with 31 per cent in the group 50 to 64 at examination. The men with marked obesity had in the aggregate a mortality 79 per cent above normal as compared with 42 per cent for those who were moderately obese.

The overweight women also had an overall mortality which was one and one-half times the expected. Among the women, as among the men, those markedly obese had a higher mortality than those who were moderately obese. In the former group the recorded mortality was 61 per cent in excess of the standard, as compared with 42 per cent in the other group.

Not only does obesity shorten life, but it is apparently an etiologic factor in several relatively common diseases. There is evidence that hypertension, cardiac disease, diabetes and gallbladder disease are appreciably more common in obese persons than in individuals of normal body weight. Obesity may adversely affect the outcome of childbearing and surgical procedures. It impairs the prognosis of persons with asthma, bronchitis, and many other conditions.

All of these facts taken together call for a re-evaluation of the term "malnutrition" when applied to the nutritional status of the citizens of this country, and indicate that the time has come for a comprehensive attack on obesity as a problem of prime importance in public health.

VITAMIN K_1 EMULSIONS IN ANTICOAGULANT THERAPY

Use of anticoagulant drugs in the management of thromboembolic states, notably coronary artery thrombosis, has been standard therapeutic practice for some time. Among such drugs, the orally administered prothrombin antagonists, dicumarol (3,3′-methylenebis (4-hydroxycoumarin); *Nutrition Reviews* **3,** *55 (1945)*), and more recently Tromexan (3,3′-carboxymethylenebis (4-hydroxycoumarin)), have proved to be most convenient and inexpensive. However, use of them is not without hazard and as their beneficial effects in the prevention of thromboembolic complications have become better defined, their dangers too are being more and more appreciated (Editorial, *J. Am. Med. Assn.* **144,** *1466 (1950)*).

Two major categories of difficulty associated with the therapeutic use of anticoagulants are frank hemorrhage and need for emergency surgery while impaired clotting of the blood is still in evidence. Severe hemorrhage may occur in patients with ulcerations of any kind in the gastrointestinal and urinary tracts regardless of how meticulously the anticoagulant therapy is controlled. Also, elderly patients and patients with liver or kidney diseases may exhibit multiple hemorrhages while they are receiving anticoagulant drugs in amounts considered safe for younger persons without renal or hepatic disorders. Idiosyncratic responses to a standard dose of an antiprothrombin drug may make control difficult even when good laboratory facilities for following the prothrombin time are available. Of course, without such facilities, anticoagulant therapy involving use of prothrombin antagonists should not be undertaken.

The potential occurrence of hemorrhagic complications makes the need for an effective, rapidly acting antidote imperative. In

the case of heparin hypocoagulability, an excellent antidote is available. Protamine sulfate given intravenously ordinarily converts prolonged clotting values (due to heparin) to normal in a matter of minutes. Similar rapid reversal of hypoprothrombinemic hypocoagulability has never been possible and presumably never will be since the production of new prothrombin by the liver always must be awaited. Blood transfusions and parenteral administration of synthetic vitamin K preparations closely related to 2-methyl-1,4-naphthoquinone (menadione, K_3; *Nutrition Reviews* **4,** *324 (1946)*) have been the only recourse of the majority of physicians confronted with bleeding emergencies due to use of prothrombin antagonists. Results with blood transfusions and the conventional synthetic vitamin K-active preparations have been largely disappointing. Accordingly, a recent report (T. B. Van Itallie, R. P. Geyer, and F. J. Stare, *J. Am. Med. Assn.* **147,** *1652 (1951)*) describing successful treatment of various emergencies arising from the use of dicumarol merits close attention.

The authors administered 5 per cent emulsions of vitamin K_1 (2-methyl-3-phytyl-1,4-naphthoquinone) in amounts ranging from 5 to 10 mg. per kilogram body weight to patients who had received dicumarol and urgently required rapid termination of the dicumarol effect. Striking results were noted after intravenous administration of the vitamin K_1 emulsions. For example, the prothrombin activity of one patient went from 24 per cent to 65 per cent in only seven hours. This patient had been under treatment for thrombophlebitis of the leg and towards the end of his hospital stay an urgent personal matter arose requiring his presence at home. Any anxiety about the state of coagulability of his blood was quickly dispelled after intravenous administration of the K_1 emulsion, and the patient was allowed to leave the hospital the same day. The authors describe another patient who required emergency surgery for intestinal obstruction while under anticoagulant therapy. Again vitamin K_1 emulsion given intravenously was associated with a rapid rise of prothrombin activity to manifestly safer levels. By contrast, a third emergency case was cited in which a conventional water-soluble vitamin K-active compound was used. Forty-eight hours after repeated injections of the K preparation the prothrombin activity had only risen from 12 to 24 per cent.

In an earlier paper on the use of vitamin K_1 emulsions in the treatment of experimental dicumarol-induced hypoprothrombinemia in the dog (M. Shoshkes, R. P. Geyer, G. S. Yee, and F. J. Stare, *J. Lab. Clin. Med.* **36,** *531 (1950)*) it was pointed out that vitamin K_1 had been regarded as an effective dicumarol antagonist for a long time (D. F. James, I. L. Bennett, P. Scheinberg, and J. J. Butler, *Arch. Int. Med.* **83,** *632 (1949)*). However, because of its physical nature (that of an oil) it had not been convenient to administer it intravenously. A. M. Seligman, A. Hurwitz, H. A. Frank, and W. A. Davis (*Surg. Gynec. Obstet.* **73,** *686 (1941)*) had accomplished this by dissolving the vitamin K_1 in alcohol and administering the solution as a highly dilute suspension in dextrose or saline solutions. Using similar suspensions, C. S. Davidson and H. MacDonald (*New Engl. J. Med.* **229,** *353 (1943)*) also reported good results in the treatment of dicumarol-induced hypoprothrombinemia. However, such suspensions were difficult to control from the standpoint of dosage and were inconvenient from the standpoint of sterilization and administration. By contrast, it was found that emulsions of vitamin K_1 could be given quickly and conveniently and in accurately measured amounts by syringe (D. M. Watkin *et al.*, *J. Lab. Clin. Med.* **37,** *269 (1951)*). Such emulsions were found to remain stable in the circulation as well as during autoclaving. Evidence for their rapid utilization was of course apparent from the prothrombin response to the emulsified vitamin. The emulsions, incidentally, were made in a manner similar to that used in the manufacture of

fat emulsions for caloric purposes (Geyer, G. V. Mann, and Stare, *Ibid.* **33,** *153 (1948)*), and were stabilized with a small amount of purified phosphatide and polyglycerol ester of oleic acid.

It may be anticipated that emulsions of vitamin K_1 will be just as effective or more so in the treatment of hypoprothrombinemia induced by some of the newer antiprothrombin drugs. They also should prove to be useful in the study of coagulation mechanisms (T. B. Van Itallie, R. P. Geyer, and F. J. Stare, *J. Clin. Invest.* **30,** *680 (1951)*) and, indeed, may introduce a new era of intravenous administration of oily and oil-soluble materials.

Since such preparations appear to combine a degree of convenience and effectiveness which presently available vitamin K preparations do not possess, it is clear that vitamin K_1 emulsions administered by the intravenous route should be the preferred treatment when a "bleeding emergency" due to an antiprothrombin drug occurs. It is hoped that these potentially life-saving emulsions soon will become generally available.

NUTRITION AND SUSCEPTIBILITY TO TUBERCULOSIS

The influence of nutritional state upon susceptibility to infection continues to be a vexing problem. This is particularly true of tuberculosis, where indirect evidence lends support to the thesis that undernutrition is associated with an increased susceptibility to the disease. The high incidence of tuberculosis in underprivileged areas of the world and particularly in semistarvation are cases in point. Nevertheless, a causal relationship is difficult to establish, since other factors such as increased exposure, inadequate housing and clothing, and overcrowding may each play a part to an unknown degree. The provision of a plentiful diet to patients with tuberculosis has become a standard of good medical practice. Extensive studies have been made of the various nutrients in patients with tuberculosis, both in patients with the disease, and, in a few instances, prior to development of the disease, in an attempt to relate deficiency of nutrients to cause.

Among the early observations was that of a blood deficiency of vitamin C and vitamin A which led to the use of the "McConkey cocktail" (cod liver oil and tomato juice) in the treatment of pulmonary tuberculosis. It was soon found that even with very large doses "saturation" with these vitamins was difficult to obtain when active disease was present. However, a little evidence has been forthcoming to support a causative relation between deficiency of these nutrients and the incidence of tuberculosis. A study in Denmark during World War I suggested a parallel relationship between tuberculosis, xerophthalmia and the unavailability of fats (*Nutrition Reviews* **5,** *97 (1947)*).

Of interest in regard to ascorbic acid and vitamin A deficiency and the development of tuberculosis is the study of H. R. Getz, E. R. Long, and H. J. Henderson (*Am. Rev. Tuberc.* **64,** *381 (1951)*). These investigators studied a relatively stable, nontuberculous but susceptible population for five years with respect to nutrition and sought to correlate previous nutritional status with the appearance of disease. The study was begun with 500 nontuberculous Negro males between the ages of 20 and 45 years. As subjects were lost from the study they were replaced by new individuals so that by the end of five years 1100 men free from pulmonary tuberculosis at the first examination had been followed for periods of from one month to five years.

At the first examination, a detailed nutritional and clinical study was made and the following measurements recorded: per cent standard body weight, erythrocyte sedimentation rate, blood hemoglobin,

plasma carotene, vitamin A, vitamin C, total serum protein, serum albumin and globulin, and serum calcium, phosphorus, and phosphatase concentrations. Roentgenographic examination of the chest was made in each patient when first seen. Unfortunately, in a number of instances, because of shortage of x-ray film this was confined to fluoroscopy so that of the 28 patients who subsequently developed tuberculosis, more than 8 had had no chest film but only fluoroscopy done before the disease became apparent. There may be a little doubt, then, that these patients did not have tuberculosis when the study was begun. However, of the 28 who developed, or were thought to have developed pulmonary tuberculosis, the values for the above observations were found to be scattered throughout the frequency distribution curves in a random fashion except for the blood concentrations of vitamin A and vitamin C. The values for these two nutrients in the 28 presumably nontuberculous individuals who subsequently developed active tuberculosis were all at the lower range of the values of the entire group studied.

Since a number of the patients had negative initial chest examinations only by fluoroscopy and since 12 others who did develop evidence of tuberculosis by chest plate had no clinical evidence of the disease, there were only 8 patients who were negative by chest film at the beginning of the study and who developed clinical tuberculosis. This makes evaluation of the study more difficult. However, the values for vitamin C in this group were with one exception all below 0.1 mg. per cent, which was the arithmetic mean for the entire group at the first test. The arithmetic mean for the entire group for vitamin A was 100 I.U. per 100 ml., whereas of the 8 patients who developed tuberculosis, the initial values of 6 were below 100 I.U. per 100 ml., the lowest being 32. There was no correlation between the deviation from standard weight or bone density and the subsequent development of tuberculosis.

This study appears to be an important one and should lead to further investigations of the significance of previous nutritional status upon the development of tuberculosis as well as other diseases. The study certainly strongly suggests that the individuals who subsequently developed tuberculosis in this group fell among those with low plasma vitamin A and vitamin C concentrations. Because of the questions raised above, the study needs elaboration and confirmation. The possible reasons for low plasma levels of these two nutrients in this group of patients were not discussed by the authors.

RIBOFLAVIN REQUIREMENTS IN PREGNANCY

Determination of the proportion of dietary riboflavin which is excreted in the urine of individuals on limited intake has been utilized in the estimation of the requirement for the vitamin. M. K. Horwitt *et al.* (*J. Nutrition* **39,** *357 (1949)*) found a marked increase in the percentage of riboflavin excreted when the daily intake was increased from 1.1 to 1.6 mg. in subjects who consumed a diet providing 2200 calories per day. It was concluded that the minimal daily requirement for this vitamin lay between these two quantities. This estimate is in good agreement with that of 0.5 mg. daily per 1000 calories which had been suggested from earlier observations (*Nutrition Reviews* **5,** *60 (1947)*). In an effort to estimate requirements in pregnancy, H. Oldham, B. B. Sheft, and T. Porter (*J. Nutrition* **41,** *231 (1950)*) determined the percentage of ingested riboflavin excreted in the urine of pregnant women and found it no less than that of women who were not pregnant. This finding was interpreted as indicating that

requirements for riboflavin are not increased in pregnancy.

This conclusion is not in accord with observations described in reports from the Rothschild-Hadassah Hospital in Jerusalem. Clinical manifestations of ariboflavinosis were found frequently in pregnant women, with greatest incidence in the last trimester of pregnancy and with regression after delivery (K. Braun, Y. M. Bromberg, and A. Brzezinski, *J. Obstet. Gynec. Brit. Empire* **52,** *43 (1945)*). Twenty-one per cent of women attending prenatal clinics exhibited manifestations of this deficiency during periods of food restriction in the years of the recent war. Clinical signs occurred most frequently in women of the lower income groups. Other members of family groups consuming the same type of diet were but rarely found to have evidences of riboflavin deficiency. Pregnancy, therefore, seemed to be associated with increased requirements for riboflavin.

In a subsequent report the same investigators describe an unfavorable effect of riboflavin deficiency on the course of pregnancy and parturition (A. Brzezinski, Y. M. Bromberg, and K. Braun, *J. Obstet. Gynec. Brit. Empire* **54,** *182 (1947)*). Two groups of women were followed throughout the course of pregnancy. In a group of 122 women whose average rate of urinary excretion of riboflavin was less than 200 micrograms per liter, the occurrence of vomiting during the second half of pregnancy, stillbirths, prematurity of birth, and inability to nurse infants was much more frequent than in another group of women whose intake of riboflavin was greater and in whom average rates of urinary excretion exceeded the low level of 200 micrograms per liter.

A third study relating to increased requirements for riboflavin in pregnancy has recently been published by the same authors (A. Brzezinski, Y. M. Bromberg, and K. Braun, *J. Lab. Clin. Med.* **39,** *84 (1952)*). The concentration of riboflavin in a single fasting specimen of urine was determined twice a month during pregnancy and the first two months of the postpartum period. Microbiologic assay was used in these determinations. One group of 29 women consumed a diet providing from 0.7 to 1.3 mg. of riboflavin daily throughout the period of study. Another group of 18 women were on a diet allowing 3.5 to 4.0 mg. daily. The women on the low level of intake had a mean concentration of the vitamin in urine during pregnancy which was identical to that of other women on the same level of intake who were not pregnant. Of greater interest was a decrease in urinary concentration of riboflavin during the last trimester. The mean value was 126 micrograms per liter in the second trimester and only 93 micrograms per liter in the third trimester, a difference which was shown to be significant by statistical test. In the first week postpartum there was a marked increase in urinary riboflavin with a mean value of 199 micrograms per liter. This was reduced to 146 micrograms during the first two months after delivery.

Average concentrations of riboflavin in urine of the women who ingested the high riboflavin diet were 419 micrograms per liter in the second trimester, 243 micrograms in the third trimester, 424 micrograms in the first week postpartum, and 277 micrograms for the first two months after delivery. In both groups the tendency toward decreased excretion of the vitamin in the latter part of pregnancy is manifest. This was explained as being due to increased requirements associated with this phase of pregnancy. In the group of women consuming diets containing larger quantities of riboflavin, excretion was significantly decreased during the two months immediately post partum. This was interpreted as evidence of an increased requirement associated with lactation.

Of particular interest was the fact that clinical manifestations of riboflavin deficiency appeared during the course of pregnancy and lactation in 19 of the 29 women

on the low riboflavin diet. Of these, 2 had first manifestations in the second trimester, 16 in the third trimester and one during lactation. Clinical manifestations included glossitis, cheilosis, angular stomatitis, seborrheic dermatitis and burning sensations in and about the mouth. These symptoms and signs were most severe during the third trimester of pregnancy. Improvement and healing occurred without provision of supplements following delivery. None of the patients on the high riboflavin diet exhibited manifestations of deficiency.

In these investigations there is impressive evidence that requirements for riboflavin are in reality increased in pregnancy and lactation. However, a number of observations deserve further comment. The manifestations which are described by the authors as signs of deficiency may not have been the result of specific deficiency. Riboflavin was not administered to the group of pregnant women who developed deficiency signs during the course of pregnancy. Rather, the skin and mucosal lesions healed spontaneously with termination of pregnancy. The diet consumed by these women is not described further than to designate its content of riboflavin. Some questions must, therefore, remain concerning the etiologic factors responsible for the manifestations exhibited.

In the carefully controlled studies of induced riboflavin deficiency in humans reported by M. K. Horwitt, C. C. Harvey, O. W. Hills, and E. Liebert (*J. Nutrition* **41,** *247 (1950)*) manifestations of deficiency did not occur when daily urinary excretions exceeded 40 micrograms. Signs of deficiency occurred in more than half of the group of women on the low riboflavin diet, who during the third trimester of pregnancy excreted the vitamin at an average concentration of 93 micrograms per liter and undoubtedly at a rate much in excess of 40 micrograms per day.

It should also be pointed out that the periods of decreased urinary excretion of riboflavin in these women correspond to periods in which nitrogen storage and utilization would be greatest, *i.e.*, the last trimester of pregnancy and during lactation. Similarly, the period of protein catabolism associated with the immediate postpartum period was a period in which excretions of riboflavin increased (*Nutrition Reviews* **10,** *58 (1952)*). These findings are in line with other findings of metabolic interrelationships between riboflavin and protein in man.

THE MONKEY ANTIANEMIA FACTOR

Workers at the University of Wisconsin have accumulated evidence to indicate that a still unidentified dietary factor is essential for the prevention of anemia in monkeys. A deficiency of this factor was noted to develop concomitantly with a deficiency of riboflavin, pyridoxine, pantothenic acid, or folacin. Refeeding of any one of these vitamins to monkeys deficient in it did not produce complete recoveries unless liver products or milk were also fed. These presumably supplied the unidentified factor (J. M. Cooperman, K. B. McCall, and C. A. Elvehjem, *Science* **102,** *645 (1945)*; Cooperman, W. R. Ruegamer, and Elvehjem, *Proc. Soc. Exp. Biol. Med.* **62,** *101 (1946)*). The anemia caused by a deficiency of this unidentified factor is characterized by a lowered blood hemoglobin, leukopenia, a reversal of the neutrophil-lymphocyte ratio, and a lack of growth.

In a recent paper, workers at the Wisconsin laboratory have reported the effects of vitamin B_{12}, folacin, and liver fractions on anemic monkeys (S. C. Smith and C. A. Elvehjem, *J. Nutrition* **45,** *47 (1951)*).

The basal diet used consisted of the following (in per cent): sucrose, 73; vitamin-test casein, 18; salts, 4; and corn oil fortified with vitamins A and D, 5 per cent. Daily

vitamin supplements, in milligrams, included the following: thiamine hydrochloride, 0.5; riboflavin, 0.5; niacin, 5.0; pyridoxine hydrochloride, 1.0; calcium pantothenate, 3.0; biotin, 0.02; choline chloride, 25; meso-inositol, 50; sodium para-aminobenzoic acid, 100; ascorbic acid, 25; and folacin, 0.1. Two monkeys were maintained in good health on this diet for a period of sixty-two weeks. Sulfasuxidine had to be administered with the vitamin supplements occasionally during the course of these studies in order to correct dysentery, a condition that was noted particularly when folacin was omitted from the diet.

Four young rhesus monkeys were fed the basal diet without the folacin supplement until the anemic syndrome had developed. Folacin, administered at a level of 0.5 mg. per monkey daily, resulted in partial recoveries, whereas daily doses of 1.0 mg. reestablished a normal blood picture and good growth. The latter dose is approximately ten times the normal daily requirement of the monkey for folacin.

Six monkeys suffering from the anemic syndrome as a result of deletion of folacin from the diet were given varying amounts of a 60 per cent methanol extract of liver. This preparation had been shown to contain an unidentified factor essential for the mink and fox (*Nutrition Reviews* **6,** *344 (1948)*). Although some improvement was obtained from this therapy, the results were neither consistent nor optimum. However, complete recoveries invariably were obtained in such monkeys by administering low levels of folacin, namely 0.1 mg. per monkey daily. The methanol extract of liver thus appeared to enhance considerably the effectiveness of folacin.

Since the liver preparation used contained some vitamin B_{12} and folacin (4 micrograms of each in the quantities of liver usually employed), the effectiveness of vitamin B_{12} alone was determined in 9 young monkeys that exhibited severe deficiency symptoms after having been on the folacin-deficient diet for fourteen to twenty-two weeks. Of the 9 monkeys in the test only 2 were improved sufficiently by vitamin B_{12} to survive. The vitamin B_{12} dosage ranged from 1 to 12 micrograms daily.

From the results, it is indicated that vitamin B_{12} is relatively unimportant in the monkey anemia produced on a folacin-deficient diet, and that either liver or high dosage of folacin is necessary for remission. Although high doses of folacin are effective, it was considered unlikely that the citrovorum factor (CF) of H. E. Sauberlich and C. A. Baumann (*J. Biol. Chem.* **176,** *165 (1948)*) would be effective since reticulogen, a good source of this factor, was earlier found to be ineffective (W. R. Ruegamer, E. M. Sporn, U. D. Register, and C. A. Elvehjem, *J. Nutrition* **36,** *405 (1948)*).

In another recent paper S. C. Smith and C. A. Elvehjem (*J. Nutrition* **45,** *583 (1951)*) report results with 11 young rhesus monkeys which were fed the basal diet deficient in riboflavin until severe riboflavin deficiency had developed. The deficiency symptoms noted were anemia, leukopenia and dermatitis. Three of the 11 monkeys responded well to daily doses of 1 mg. of riboflavin and evinced no need for any additional dietary factor. Eight of the monkeys, however, failed to respond completely to riboflavin alone, but exhibited complete remissions when either ten times the daily requirement of folacin (1 mg. daily) or methanol extract of liver was also administered.

The Wisconsin investigators postulate that the intestinal flora may assume importance in the anemic syndrome in monkeys and that the high levels of folacin stimulate the intestinal synthesis of the unidentified antianemia factor.

These results emphasize that the deficiency of any one of several B-vitamins often can produce derangements in the overall functional capacity of the animal to such an extent that subsequent feeding of that

vitamin will not correct the deficiency. In such cases there is apparently a need for the still unidentified factor. A deficiency of the unidentified factor apparently does not occur when all the known B-vitamins are supplied in the diet.

VITAMIN B_{12} AND GROWTH OF PREMATURE INFANTS

Since vitamin B_{12} has been shown to increase the growth rate of the young of certain domestic farm animals, studies on the effect of this vitamin on the growth of human infants, especially those born prematurely, are of particular interest. It might be expected that the premature human infant who grows rapidly and whose fetal store of nutrients might be insufficient would readily respond to such supplementation. D. F. Downing (*Science* **112,** *181 (1950)*) administered vitamin B_{12} to 25 premature infants in doses of 10 micrograms intramuscularly at intervals of one to three days in total amounts averaging 70 micrograms per infant. Twenty-three infants of similar size and receiving the same feedings served as controls. Growth rates in both groups were the same.

A more extensive study has been reported by H. Rascoff, A. Dunewitz, and R. Norton (*J. Pediat.* **39,** *61 (1951)*). These workers fed 5 micrograms of vitamin B_{12} daily to a group of 40 premature infants and continued their observations for four to six months. The weight gain from the seventh to twenty-eighth day was comparable to that of a larger group of patients previously studied in the same hospital. The weight gains during the two- to four-month and four- to six-month intervals were not significantly different from those reported by K. Glaser, A. H. Parmelee, and E. B. Plattner (*Pediatrics* **5,** *130 (1950)*). The same amount of vitamin B_{12} was given to the smaller of each of 7 sets of twins. In only one instance did the treated infant surpass his control twin in weight gain. Increase of the daily oral dose of vitamin B_{12} to 20 micrograms in a later series of 25 premature infants was not associated with increased growth.

Both vitamin B_{12} and folic acid have been given to premature infants by T. Mitchell, J. N. Etteldorf, A. H. Tuttle, and G. W. Clayton (*Pediatrics* **8,** *821 (1951)*). Both vitamins were administered parenterally during the first 50 days of life. No significant differences in weight gain were found between a control group and groups of infants receiving 10 micrograms of vitamin B_{12} daily, weekly, daily in combination with 6 mg. of folacin, or 6 mg. of folacin alone. Determinations of the hemoglobin concentration, red blood count and hematocrit were carried out at suitable intervals and the blood indexes were calculated. No differences between the groups were noted. The hematologic data included in this report are of considerable interest because anemia is one of the principal manifestations of deficiencies of both of these vitamins in the human adult.

Since vitamin B_{12} appears to have no stimulating effect on the growth of premature infants, one may be justified in assuming that these infants are adequately supplied with this vitamin either by tissue reserves or from their food. The production of vitamin B_{12} deficiency in the newborn rat is possible only when the maternal organism is depleted by feeding a diet devoid of this factor (*Nutrition Reviews* **9,** *310 (1951)*). All of the infants studied received formulas consisting of modified cow's milk mixtures which are considered to contain adequate quantities of both vitamin B_{12} and folacin to meet the presumed requirements of full term infants (*J. Am. Med. Assn.* **146,** *1028 (1951)*). Consequently, the failure of vitamin B_{12} to accelerate the growth of premature infants should not be unanticipated.

DIET AND INSULIN EFFECT ON DIABETES AND CATARACT DEVELOPMENT IN RATS

The factors involved in the development of the complications of diabetes are still poorly understood. In the experimental animal made diabetic by alloxan or by pancreatectomy, cataracts, renal lesions, and in lesser incidence, vascular lesions, have been described (F. D. W. Lukens, *Proc. Am. Diabetes Assn.* **10,** *103* (*1950*)). The influence of dietary constituents and control of hyperglycemia by insulin on the development of these complications has been the subject of investigation in numerous studies. However, species variations have prevented the formation of broad conclusions. A recent attempt to delineate the relative influence of carbohydrate, protein, and fat, and the control of hyperglycemia on the appearance of cataracts in the diabetic rat has been reported by R. R. Rodriguez and W. A. Krehl (*Yale J. Biol. Med.* **24,** *103* (*1951*)).

By the technics of 95 per cent pancreatectomy and alloxan injection the production of diabetes was attempted in groups of rats of Yale and Wistar strains respectively. The incidence of diabetes in the pancreatectomized rats was first studied in groups receiving synthetic isocaloric diets of three types: high carbohydrate, high protein, and high fat. The animals were allowed to eat ad libitum and food intakes were calculated at intervals, although no data were recorded by the authors for the various regimens. Two additional groups of rats were fed high carbohydrate diets, one with added cholesterol and another without choline. After six months the incidence of diabetes in animals fed these diets was 80 per cent for the high carbohydrate-, 83 per cent for the high fat-, and 33 per cent for the high protein-fed animals. The other two diets produced 65 and 66 per cent diabetes respectively. The protective effect of protein in pancreatectomized rats is thus apparent, substantiating a previous report of B. A. Houssay and C. Martínez (*Science* **105,** *548* (*1947*)). Unfortunately, the study reviewed here is without data concerning the possible variations in quantities of the various diets actually consumed. The factor of varying caloric intake cannot therefore be evaluated in explaining the results obtained.

Rodriguez and Krehl then compared the incidence of cataracts in the pancreatectomized rats with that in the alloxan-treated rats after six months of diabetes. In groups of animals of each type receiving the three major diets the incidence of cataracts was significantly higher in the alloxan-injected animals receiving carbohydrate and protein diets (80 and 20 per cent respectively) than in pancreatectomized groups receiving the same diets (38 and 0 per cent respectively). By comparison, no animals in either group developed cataracts when high fat diets were received. There was a rather good correlation of the incidence of cataracts with the degree of glycosuria displayed by the variously treated groups. The average urinary glucose in grams per rat per day was highest in the alloxan-high carbohydrate animals (5.13 g.) compared with similarly fed pancreatectomized animals (2.41 g.). High protein feeding yielded urinary glucose values of 2.27 g. in the alloxan group versus 0.85 g. in the pancreatectomized group. High fat diets, however, yielded only 0.27 and 0.37 g. per day in the alloxan and pancreatectomized groups respectively.

A second series of pancreatectomized animals fed high carbohydrate diets was given insulin with reduction in glycosuria to 0.72 g. per day. No cataracts occurred. The exceptions to the correlation of glycosuria and cataract incidence were found in the pancreatectomized animals which received carbohydrate diets altered with added cholesterol or without choline. Glycosuria of 2.44 and 2.12 g. per day was found in these groups, figures comparable to the

glycosuria in the unaltered high carbohydrate animals, yet cataracts occurred in only 18 per cent of the animals in both of these groups. The authors state that the lower incidence of cataracts in these two groups may have been due to somewhat lower daily food intakes, but no explanation of the similar degree of glycosuria is offered.

The evidence offered in this series of studies is suggestive of the effect of protein in protecting experimental animals such as these against the development of diabetes. The degree of glycosuria and the frequency of cataract development also appear to be correlated, except for the instances noted. The unquestioned acceptance of the hypothesis of hyperglycemia as a primary cause of cataract development, however, will await extension of this type of study and explanation of the discrepancies noted. Even greater caution in relating these observations in rats with experimental diabetes to the disease in humans should be exercised in view of the variation in the ease with which cataracts can be produced among species of animals (Lukens, *loc. cit.*).

ARTERIOSCLEROSIS IN THE GOLDEN HAMSTER

Experimentally produced arteriosclerosis has been observed in several animal species including the rabbit, chicken, dog and golden hamster (*Nutrition Reviews* **9,** *295 (1951)*). In the golden hamster it was previously noted that abnormalities occurred in the liver which were considered to be Kupffer cells loaded with cholesterol. Some additional information on arteriosclerosis as well as on the liver changes has been furnished by studies reported on by W. Marx, L. Marx, and H. J. Deuel (*Am. Heart J.* **42,** *124 (1951)*). Twenty-eight adult golden hamsters of varying ages were fed ad libitum a diet consisting of one part stock rat diet, one part Sherman diet, one part rolled oats, and a supplement of lettuce two times weekly. The stock rat diet consisted of 34 per cent whole wheat flour, 34 per cent oats, 15 per cent skimmed milk powder, 4 per cent alfalfa meal, 2 per cent yeast, 8 per cent cottonseed oil, 2 per cent oil fortified to contain 300 I.U. of vitamin A and 50 I.U. of vitamin D per gram, 0.5 per cent sodium chloride and 0.5 per cent calcium carbonate.

Nine animals were fed this diet and nothing in addition. Six animals were given 1 per cent cholesterol which was dissolved in hot cottonseed oil and mixed with the diet. Thirteen animals were given the cholesterol to which U.S.P. dessicated ox bile was added in an effort to enhance the absorption of the cholesterol. The animals were kept on the diets for from sixteen to twenty-five weeks, at which time they were killed, autopsied, and the plasma and livers analyzed for cholesterol.

At autopsy it was observed that the livers had increased markedly in size and were pale yellow. This was particularly true in the group given bile in addition to cholesterol. Analyses of the plasma of these animals showed a marked increase in cholesterol from an average of 52 mg. per cent in the normals to 162 mg. per cent in those fed cholesterol alone, and 282 mg. per cent in the animals fed cholesterol and bile. Likewise, there were great increases in the liver cholesterol content. In the animals fed cholesterol plus bile the liver cholesterol was as high as 12 per cent, with an average liver cholesterol content of 5.11 g. per 100 g. This figure was compared with the cholesterol content of the livers of the animals not given cholesterol, which was 0.22 g. per 100 g.

Grossly, the aortas appeared normal in all animals whether fed cholesterol, cholesterol and bile, or without added cholesterol. Histologically, no fat was observed in the aortas of the untreated animals when they were stained with Nile blue sulfate. How-

ever, 4 of the cholesterol-fed hamsters did exhibit fatty imbibition, with the endothelium loaded with fat and foam cells.

The authors note that their results differ somewhat from those of others who have attempted to produce arteriosclerosis in the golden hamster, and feel that it depends in part upon the basic diet and also in this instance upon the addition of bile to the cholesterol-containing diet. They also point out that there appears to be a poor correlation between species of animals as to the cholesterol content of the plasma, livers and aortas.

VITAMIN E ACTIVITY OF METHYLENE BLUE

Considerable effort has been devoted, during the past decade, to the search for the primary biochemical lesion in vitamin E deficiency. Previous reviews (*Nutrition Reviews* **3,** *17* (*1945*); **6,** *346* (*1948*); **8,** *135* (*1950*)) have discussed the main conclusions of experimental work in this field. In particular, it has been shown that while certain *in vitro* respiration studies seemed to indicate that the normal action of vitamin E is an inhibition of succinic acid dehydrogenase, it appears probable that the results can best be interpreted on the basis of a nonspecific action of the various tocopherol phosphates and an equally nonspecific surface action by vitamin E or by unrelated substances. The problem of the biochemical role of vitamin E remains, therefore, as intriguing as ever. However, a series of recent papers by H. Dam and his collaborators demonstrates that a number of substances and, in particular, that long-known and oft-described model of oxidation-reduction agents, methylene blue, exhibit considerable vitamin E activity, being capable of replacing the vitamin in a variety of physiologic processes in several animal species.

Although the most classic signs of vitamin E deficiency in laboratory animals are no doubt familiar to the reader, it may be useful to list the most characteristic symptoms observable in the rat and the chick, the two experimental animals used by the Copenhagen workers. In the rat, vitamin E deficiency causes sterility in both the male and female. In the male, it produces irreversible sterility characterized by degeneration of the germinal epithelium; in the female it causes degeneration and often resorption of the fetus. Feeding rats diets deficient in vitamin E and containing cod liver oil also causes a typical depigmentation of the incisors. Depot fats are abnormally brown and contain a large amount of peroxides. A combination of low protein content and absence of vitamin E in the diet causes rats to develop massive liver necrosis and lung hemorrhage. The blood of vitamin E-deficient rats is hemolyzed, both *in vitro* and *in vivo*, by contact with dialuric acid. Finally, rats clearly exhibit the classic "sparing effect" of vitamin E on vitamin A stores in the liver.

Vitamin E has been postulated to be an antioxidant protecting vitamin A from oxidation, both in depot fats and in the alimentary tract (T. Moore, *Biochem. J.* **34,** *1321* (*1940*); K. C. D. Hickman, P. L. Harris, and M. R. Woodside, *Nature* **150,** *91* (*1942*)). Vitamin E deficiency causes secondary destruction of vitamin A stores, and if these are limited in amount the vitamin E-deficient rats may exhibit a secondary vitamin A deficiency. As it has been shown (H. Dam, *J. Nutrition* **27,** *193* (*1944*)) that symptoms of vitamin E deficiency are influenced in frequency, type and severity by the kind and amount of unsaturated fatty acids contained in the diet, it becomes understandable that this destruction of vitamin A stores seems to be influenced in turn by the nature of the fat of the diet.

In the chick, the most characteristic signs of vitamin E deficiency are an alimentary

exudative diathesis (a predisposition to subcutaneous inflammatory changes with swelling of lymph glands) and encephalomalacia (a disease of the brain tissue). In addition, vitamin E seems to function in this species too as the chief antioxidant of adipose tissue: the fat of deficient chicks shows a brown coloration similar to that observed in deficient rats, particularly if the diet contains generous proportions of cod liver oil. This coloration is again associated with a high peroxide value. Growth is impaired. Stores of vitamin A in the liver are diminished, particularly if the diet is high in unsaturated fats.

In the first experiments described by Dam and his collaborators (H. Dam, I. Kruse, I. Prange, and E. Søndergaard, *Acta physiol. Scandinav.* **22,** *299* (*1951*)) chicks were used as the experimental animal. The exudative diathesis and encephalomalacia characteristic of vitamin E deficiency were noted in chicks fed unsupplemented diets. Growth was also markedly depressed on the deficient diets. Mortality was high. A variety of substances, known to have marked antioxidant properties, were tested for the possible protection afforded against these two conditions. It was found that marked protection against exudative diathesis, as judged by delayed incidence and reduced mortality, was given by very small supplements (of the order of one part per thousand) of methylene blue, thionine, thiodiphenylamine or antabuse. Supplements of 0.5 per cent ascorbic acid were also effective against this symptom. On the other hand, while methylene blue gave good protection against encephalomalacia, other supplements, antabuse in particular, were ineffective. Methylene blue, thionine and thiodiphenylamine also caused a marked improvement of the growth of the vitamin E-deficient chicks. The response was, in fact, entirely similar to that given by administration of alpha-tocopherol acetate. Finally, the appearance of the adipose tissue (absence of browning) as well as peroxide values obtained in depot fat demonstrated that antabuse, methylene blue and thionine also afforded considerable protection against the occurrence of a high concentration of peroxide in the depot fat of the deficient animals.

The protective action of these substances, methylene blue in particular, against exudative diathesis and encephalomalacia being so clear cut, Dam and his collaborators pursued the analogy with the action of vitamin E in the chicks by investigating the effect of adding these substances to a vitamin E-free diet on liver storage of vitamin A (H. Dam, I. Prange, and E. Søndergaard, *Acta pharm. et toxicol.* **8,** *1* (*1952*)). When chicks were fed vitamin E-deficient diets containing 10 per cent of cod liver oil, methylene blue, thiodiphenylamine and antabuse all caused an increased deposition of vitamin A in the liver and significantly increased the growth rate. The effect was entirely comparable to that of alpha-tocopherol or its acetate added under the same conditions. Replacement of the cod liver oil of the diet by lard demonstrated in another experimental series that the nature of the dietary fat was also important with these substances. In this case neither methylene blue nor tocopherol acetate had any effect on growth or on vitamin A storage. The livers of the animals of this series, in opposition to those of the previous series, still contained appreciable amounts of vitamin A at the completion of the experiment. The authors therefore concluded that the protective action of vitamin E and of its substitutes had consisted not of a direct action on vitamin A, but of one on the detoxification of some of the components of the cod liver oil which would otherwise decrease both vitamin A stores and growth rate, possibly by causing a certain degree of liver damage. This explanation seems consistent with the concept already mentioned, and largely due to Dam, that the severity of vitamin E deficiency is a function of the

content of the diet in easily autoxidizable unsaturated fatty acids.

An attempt was made to simulate *in vitro* the conditions of digestion in the glandular and muscular stomachs to see if vitamin A was affected by vitamin E in gastrointestinal processes as well as in liver storage. No such effect was found during this "digestion" and the authors concluded that vitamin E and the related substances do not exert their protection on vitamin A stores by effects on the digestive processes. The protection would thus appear to be essentially hepatic.

In the rat, the first phenomenon studied was the inhibitory effect of dietary methylene blue on dialuric acid hemolysis of erythrocytes from vitamin E-deficient animals (F. Christensen and H. Dam, *Acta pharm. et toxicol.* **7,** *167* (*1951*)). The diet used contained 38 per cent lard and 2 per cent cod liver oil. The massive hemolysis observed when no supplement was included in the diet was almost entirely suppressed when 0.126 per cent of methylene blue was added. The protective effect was almost as marked as that of a liberal amount of alpha-tocopherol acetate.

The next study (E. Aaes-Jørgensen, H. Dam, and H. Granados, *Acta pharm. et toxicol.* **7,** *171* (*1951*)) dealt with the influence of antabuse and methylene blue on changes in the adipose tissue and incisors of rats receiving high (10 or 20 per cent) cod liver oil, vitamin E-deficient diets. The color of both adipose tissue and that of the incisors was graded according to well-established scales. Peroxide values of samples of lumbar fat were determined. It was found that both antabuse and methylene blue afforded good protection against depigmentation of the incisors and coloration of the adipose tissue, equal or, in some cases, superior to that observed with vitamin E. Peroxide values were drastically decreased, antabuse being at least as effective as methylene blue, and both substances gave rise to reductions of the same order of magnitude as that given by tocopherol acetate. On the other hand, methylene blue caused some reduction in the growth rate; this effect was less marked with antabuse.

Experimenting next with the massive hepatic necrosis and lung hemorrhage reported by P. György (*p. 34*) and K. Schwarz (*p. 45*, "Liver Injury," *Tr. 8th Conf. Josiah Macy Jr. Foundation, New York* (*1949*)) and H. P. Himsworth ("The Liver and Its Diseases," *2d edition, Harvard University Press, Cambridge* (*1950*)) to occur on low protein, vitamin E-deficient diets, H. Dam and H. Granados (*Acta pharm. et toxicol.* **7,** *181* (*1951*)) showed that these symptoms failed to appear if the diet was free of fat. On the other hand, the syndrome appeared when lard, and even more so, when cod liver oil, was incorporated in the diet. Addition of methylene blue (0.126 per cent) resulted in complete protection, and was in fact superior to addition of tocopherol.

The effect of methylene blue and thiodiphenylamine on liver storage of vitamin A was studied (H. Dam, I. Prange, and E. Søndergaard, *Experientia* **7,** *184* (*1951*); *Acta pharm. et toxicol.* **8,** *23* (*1952*)) in a manner comparable to similar studies in the chick. Some of the vitamin E-deficient diets were fat-free, others contained varying levels of cod liver oil or lard. Again it was found that when either methylene blue, thiodiphenylamine or tocopherol were added to the vitamin E-deficient diets containing cod liver oil, a marked increase in vitamin A stores could be noted, with methylene blue the most effective protective agent. No effect was noted with any of these substances on fat-free or lard-containing diets.

Finally, the oldest classic vitamin E deficiency symptom, sterility in the rat, was studied (H. Dam and H. Granados, *Acta pharm. et toxicol.* **8,** *47* (*1952*)). The possible replacement of vitamin E by methylene blue as an antisterility factor was studied in females. Various deficient diets were used, some fat-free, some containing either lard or cod liver oil as sources of fat. Methylene

blue was again found to depress growth somewhat, a result at variance with that obtained in chicks, where significantly increased growth rates were obtained on diets containing cod liver oil. However, methylene blue improved markedly, and to about the same extent as tocopherol acetate, the much-impaired reproductive capacity of female rats reared on vitamin E-deficient, fat-free and cod liver oil-containing diets. The slighter impairment of reproductive capacity observed in the groups reared on lard-containing diets was also relieved by addition of methylene blue, again to the same extent as by the administration of tocopherols.

The importance of these findings to our understanding of the physiology of vitamin E is obvious. In almost all respects the substances tested by Dam and his collaborators, methylene blue in particular, demonstrate an action strikingly similar to that of vitamin E. As the oxidation-reduction property of methylene blue has been exhaustively studied, a host of well-established findings concerning zones of oxidation-reduction potentials becomes relevant to the biochemistry of vitamin E. It would appear dangerous, however, to conclude from these studies that methylene blue "replaces" vitamin E in its characteristic chemical reactions. While a simple explanation of the findings presented here might be that methylene blue (among other substances) has similar properties as an antioxidant to vitamin E and is interchangeable with the vitamin in this capacity, H. Dam and H. Granados (*Acta pharm. et toxicol.* **8,** *47 (1952)*) point out that another interpretation might be that methylene blue replaces certain enzymatic systems normally dependent on vitamin E for their continued integrity. The fact that in rats methylene blue presents a somewhat depressing effect on growth not exhibited by vitamin E is viewed by them as possible evidence for the idea that methylene blue does not always substitute for vitamin E in the same fashion. If true, this would further complicate the interpretation of the possible replacement of vitamin E by the various oxidation-reduction substances. One wonders, however, whether this growth-depressing effect of methylene blue on growth in the rats, the only exception to the general phenomenon demonstrated by the Copenhagen workers, is not due to a slight toxic effect of methylene blue in that species, unrelated to its vitamin E activity.

The results of Dam and his collaborators are of an importance which transcends even that of the problems raised by vitamin E deficiency. In particular, it seems difficult, when considering the apparent structural diversity of the various vitamin E-active compounds, not to reconsider the whole concept of biochemical specificity and wonder what characteristics of molecules, in the last analysis, determine their biologic properties.

INTERCONVERSION AMONG NONESSENTIAL AMINO ACIDS

One of the important contributions to nutritional biochemistry during the present century has been the demonstration that ten of the amino acids resulting from the hydrolysis of proteins are indispensable for the early rapid growth of young rats. Likewise, it has been shown that for maintenance of adult rats only nine of these so-called "essential" amino acids are required (R. W. Wissler *et al.*, *J. Nutrition* **36,** *245 (1948)*), and for the maintenance of nitrogen balance in young adult human subjects only eight are necessary (W. C. Rose, *Fed. Proc.* **8,** *546 (1949)*). During the past two decades information has been secured which indicates that the nonessential amino acids play an important part in nutrition and indeed, that nonprotein nitrogenous compounds

can assume the function of a portion of these amino acids (D. V. Frost and H. R. Sandy, *J. Biol. Chem.* **189,** *249* (*1951*)).

A recent study (W. C. Rose, W. W. Burr, and H. J. Sallach, *J. Biol. Chem.* **194,** *321* (*1952*)) has turned attention to the possible influence upon growth of three nonessential amino acids, namely, glycine, serine and cystine, under conditions of dietary stress, and to the great ability of the organism to synthesize them. As pointed out, these three amino acids, in addition to their usual functions in the body, have been shown to bear unusual relationships to each other and to other compounds of biologic significance. Serine is the nitrogenous base in one component of the phospholipids isolated from brain (J. Folch, *Ibid.* **146,** *35* (*1942*)). Again, serine has been shown to be the precursor of the amino ethyl alcohol in cephalin (DeW. Stetten, *Ibid.* **144,** *501* (*1942*)), and thus of choline (Stetten, *Ibid.* **140,** *143* (*1941*)). Also, with homocysteine, serine is known to enter into the biosynthesis of cysteine (F. Binkley and V. du Vigneaud, *Ibid.* **144,** *507* (*1942*); du Vigneaud, G. W. Kilmer, J. R. Rachele, and M. Cohn, *Ibid.* **155,** *645* (*1944*)). Isotopic studies have indicated that serine is converted to glycine (D. Shemin, *Ibid.* **162,** *297* (*1946*)), and that glycine can give rise to serine in the body (W. Sakami, *Ibid.* **176,** *995* (*1948*)). Glycine can also be converted to amino ethyl alcohol directly (Stetten, *Ibid.* **140,** *143* (*1941*)), or by way of serine (M. Levine and H. Tarver, *Ibid.* **184,** *427* (*1950*)).

Although serine, cystine and glycine are known to be dispensable for rapid growth and for maintenance, their close association in the interconversion of compounds of greatest importance in intermediary metabolism suggested that conditions of nutritive stress might be imposed upon experimental animals so that the speed and even the continued ability of biosynthesis might be evaluated (Rose *et al.*, *J. Biol. Chem.*, *loc. cit.*). Weanling rats (15 animals in each experimental group) were given a purified diet adequate for growth, the nitrogen of which was provided by pure amino acids, one or more of which could be removed and the effects then observed. Thus, the influence of the removal of cystine alone was compared with that of exclusion of both cystine and serine. Also, in view of the demonstrated involvement of serine and glycine in choline formation, a low-choline diet was compared with a ration deficient in choline and lacking serine and glycine entirely. No difference in growth was observed in any of these tests.

Another comparison was made between rats given the adequate control ration and a group restricted to a diet low in choline and devoid of glycine, serine and cystine. Again, no statistically valid difference in growth was observed. The basal diet was then adjusted to contain a minimum amount of methionine for growth, a slightly deficient quantity of threonine and no choline. When the growth of rats on this diet (as a control) was compared with that when cystine, glycine and serine were all omitted, there was a significant decrease in body weight at the end of the thirty-six day experimental period. It is apparent that when these extreme dietary adjustments were made, the ability of the organism to synthesize the amino acids in question was overstepped and they then became limiting factors in growth. In view of the prevention of the formation of choline under these dietary limitations (restriction of methionine as a source of methyl groups), the fact that there is evidence of great persistence of synthesis of the four compounds omitted from the ration (cystine, serine, glycine and choline), and the more or less central position of serine in the interconversions discussed earlier, it appears that serine must be formed in the organism from something other than glycine. These studies emphasize again the relative nature of the concept of indispensability in nutritional biochemistry.

FAT IN NUTRITION

Workers at the University of Southern California have participated with others in a series of studies designed to increase our knowledge of the effect of the fat level in the diet on general nutrition. Although it is generally concluded among human nutritionists that the diet should contain approximately 30 per cent of the total calories in the form of fat, there is actually very little sound data to support such a conclusion. There are many gaps in our knowledge of the effect of fat on general nutrition. For example, the essential fatty acid content of various fats is not accurately known. Chemical methods are at best a crude estimate since they generally determine only the relative proportion of mono-, di- and tri-double-bond (enoic) fatty acids. Furthermore, only six unsaturated fatty acids have been reported to possess essential fatty acid activity whereas at least seventeen such compounds were recently reported from the California laboratory to be devoid of biologic activity.

Recently H. J. Deuel, S. M. Greenberg, L. Anisfeld, and D. Melnick (*J. Nutrition* **45,** *535 (1951)*) have reported the essential fatty acid content of margarines, shortenings, butters, and cottonseed oil as determined by two methods, spectrophotometric and biologic assay.

The spectrophotometric method was that of B. A. Brice, M. L. Swain, B. B. Schaeffer, and W. C. Ault (*Oil and Soap* **22,** *219 (1945)*). The bioassay was developed in the the California laboratory and reported in detail by S. M. Greenberg, C. E. Calbert, E. E. Savage, and H. J. Deuel (*J. Nutrition* **41,** *473 (1950)*). This assay is based on the observation that the increase of body weight in male rats, previously depleted of essential fatty acids, is a linear function of the logarithm of the dosage of essential fatty acid (linoleic acid). The growth obtained when the fat-deficient diet was supplemented with known amounts of the test fats was compared with the reference growth curve obtained on the same diet supplemented with methyl or ethyl linoleate at a level to supply 0, 5, 20 and 50 mg. per day per rat. Reference data were also obtained when the diet contained supplementary dosages of 250 and 500 mg. of hydrogenated coconut fat per rat daily. This fat, which contains no essential fatty acids, was fed to compensate for the equivalent amounts of test fats fed.

The growth response obtained when margarines were fed was generally superior to that obtained with butter, and spectrophotometric analyses indicated the essential fatty acid content of the margarines ranged between 4.4 and 5.5 per cent compared with values of 3.1 and 3.8 per cent for the 2 butter samples analyzed. In general, good agreement was obtained between the bioassay and the spectrophotometric method in the case of butter, margarine, cottonseed oil and a shortening prepared by selective hydrogenation. An unexplained result was the superior bioassay activity of shortening prepared by nonselective hydrogenation. In this case spectrophotometric analysis indicated only about one-half the activity found in bioassay. Under selective hydrogenation linoleic acid is preferentially converted to oleic and iso-oleic acids rather than to oleic and stearic acids. Selectively hydrogenated shortenings are preferred for frying purposes whereas nonselectively hydrogenated shortenings are preferred for baking.

Based on spectrophotometric analysis, selectively hydrogenated shortening was found to contain 2.1 per cent of essential fatty acids whereas nonselectively hydrogenated shortening contained 6.8 per cent. Growth responses of fat-depleted rats indicated, however, that the nonselectively hydrogenated shortening contained as much as 13.0 per cent of essential fatty acids,

contrasted with only about 2.5 per cent in the selectively hydrogenated shortening. The essential fatty acid content of cottonseed oil obtained by bioassay, 48 per cent, was approximately the same as that obtained spectrophotometrically, namely, 49.5 per cent.

The bioassay procedure indicated that the optimum level of linoleic acid required by fat-depleted male rats was in excess of 200 mg. daily, and that the requirement increased when the diet contained fat. The optimum level for female rats was found to be considerably lower than that for male rats.

In another paper, these workers reported the results of a comparison of the potency of arachidonic and linoleic acids in furnishing the requirement for essential fatty acids in the rat (S. M. Greenberg, C. E. Calbert, H. J. Deuel, and J. B. Brown, *J. Nutrition* **45,** *521 (1951)*). Arachidonic acid was found to possess about three and one-half times the activity of linoleic acid when each was fed alone. When low levels of linoleic and arachidonic acid were fed together, they produced greater growth responses than equal levels of either fed alone. Under these conditions the potency of arachidonic acid was calculated to be approximately six and two-tenths times that of linoleic acid.

The superior potency of arachidonic acid is understandable since it is generally agreed that the other essential fatty acids, linoleic and linolenic, are converted to arachidonic acid in the animal. That arachidonic acid when fed with linoleic acid is more potent than when fed alone, is, however, less readily understood. This observation, nevertheless, does emphasize that biologic assay is perhaps the only reliable means of establishing the essential fatty acid potency of fats.

In another paper (L. Anisfeld, S. M. Greenberg, and H. J. Deuel, *J. Nutrition* **45,** *599 (1951)*) the influence of dietary vitamin E on the response of fat-depleted rats to methyl linoleate was reported. A synthetic low-fat diet containing 20 per cent of casein and a synthetic vitamin mixture was fed to weanling rats during the fat-depletion period. Alpha-tocopherol was supplied during the depletion period at a level of 0.1 mg. per rat daily. When the body weights had remained constant for three weeks, they were considered to be depleted of essential fatty acids. Fifteen groups of male and 14 groups of female rats (6 to 8 rats per group) were then fed various combinations of methyl linoleate and synthetic alpha-tocopherol or D-alpha-tocopherol acetate. Methyl linoleate dosages tried were 0, 5, 20, and 100 mg. per day per rat whereas tocopherol levels tried were 0, 0.25, 0.50, and 1.0 mg. per day per rat.

Growth responses to methyl linoleate were not influenced by the additional supplement of alpha-tocopherol. This was true at all levels of methyl linoleate and alpha-tocopherol tested and is in sharp contrast to the observation by E. L. Hove and P. L. Harris (*J. Nutrition* **31,** *699 (1946)*). These workers reported a rather striking sparing action of tocopherol on the essential fatty acid requirement when it was combined with methyl linoleate in fat-depleted rats. Hove and Harris further noted that tocopherol supplements alone were without any effect on growth in such fat-depleted rats. There is one important difference in the procedures employed by these two groups of workers. The California workers supplemented the depletion diet with 0.1 mg. of tocopherol per rat daily, whereas Hove and Harris fed a tocopherol-low diet during the depletion period. It is possible that the sparing effect of tocopherol on the essential fatty acids can be demonstrated only when the fat-depleted rat has been maintained on a low-tocopherol regimen.

These divergent results again serve to emphasize that much reliable data on the interrelationships of fat-soluble essential factors in nutrition are still to be desired.

PREVENTION OF HEMORRHAGE BY RUTIN

Rutin, one of the substances in the general class of flavones thought to have a beneficial effect upon capillary permeability and fragility, has been used in the past in the treatment of conditions in which capillary changes are prominent, such as diabetic retinopathy and radiation damage. Studies with rutin have been made both in man and in experimental animals (*Nutrition Reviews* **6,** *301 (1948)*; **7,** *317 (1949)*; **9,** *106 (1951)*). In dogs in which malignant hypertension is induced, hemorrhage in various parts of the body, especially the heart, is one of the serious complications and causes of death. The effect of rutin upon animals subjected to this experimental procedure has been studied by H. K. Hellerstein *et al.* (*Am. Heart J.* **42,** *271 (1951)*). Seventeen dogs with an average weight of 12 kg., starved for twenty-four hours postoperatively, had both their renal arteries constricted by thread while anesthetized by intravenous pentobarbital. One dog died of shock eight hours postoperatively and was not included in the series. The remainder were followed postoperatively and were allowed to eat food and water. The dietary intake of the dogs during the postoperative period was not presented by the authors.

The sixteen dogs were divided into four groups. The first group of 5 dogs was given no rutin. The second group consisting of 2 dogs was given 200 mg. of rutin postoperatively per day. Each dog in the third group of 4 was given 200 mg. of rutin per day for three days preoperatively. This dosage was continued throughout the postoperative period. The fourth group of 5 dogs was given 200 mg. of rutin per day for ten days, preoperatively. This dosage was continued during the postoperative period until death.

All animals developed pathologic evidence of malignant hypertension and uremia. The blood urea nitrogen rose to levels of 200 to 300 mg. per cent, and the animals showed lassitude, anorexia and finally convulsions. All died. Eight of the dogs which had previously been trained for the procedure had their blood pressure measured by the Hamilton manometer. Seven had severe hypertension while the eighth died sixty hours postoperatively, presumably before hypertension could occur.

There was no significant difference in the survival time of the dogs in the four groups. However, at post mortem the gross massive hemorrhages in the hearts of all dogs in groups 1, 2, and 3 were not seen in group 4. Microscopically, hemorrhages into the myocardium, myocarditis and necrosis were seen to be much more marked in groups 1, 2, and 3 than in group 4. Necrotizing arteriolitis was observed in all dogs, scattered here and there throughout the body except in the kidneys which did, however, show infarctions and extensive degeneration. Marked constriction of the renal arteries was of course observed post mortem as well.

The authors concluded that rutin exerted some kind of a nonspecific stabilizing effect on the ground substance of the dogs to whom this substance was administered both preoperatively and postoperatively, especially if given long enough (ten days) preoperatively. They felt that they were unable to interpret the effect of rutin on the ground substance, but postulated from their results and those of others that this was the most likely effect of the material administered.

FOOD INTAKE AND NOCTURNAL DIURESIS IN SPRUE

The occurrence of increased urine volumes during the night hours has been noted in patients with heart and kidney disease (D. S. Baldwin, J. H. Sirota, and H. Villar-

real, *Proc. Soc. Exp. Biol. Med.* **74,** *578 (1950)*; A. M. Fishberg, "Hypertension and Nephritis," *4th edition, Lea and Febiger, Philadelphia (1939)*), in contrast to the usual daytime maximum urine excretion found in normal persons. A similar situation has been noted in patients with sprue, and the mechanism of this nocturnal diuresis has been investigated by E. E. Wollaeger and B. H. Scribner (*Gastroenterology* **19,** *224 (1951)*).

Nine patients with the typical physical and laboratory findings of nontropical sprue were studied by these workers. Of the total number, 7 showed a definite increase in night urine volume over the volume excreted during the daytime. The remaining 2 subjects had findings which were only suggestive of a nocturnal diuresis. Four of the patients were investigated under controlled conditions while receiving constant diets containing 57 g. of fat, 133 g. of protein, and 349 g. of carbohydrate per day. The total daily fluid intake was 3730 cc., of which 895 cc. was contained in the solid food. All food and fluids were given between the hours of 8 a.m. and 8 p.m., with meals being served at regular hours and between-meal feedings given at 10 a.m., 3 p.m. and 8 p.m. Urine collections were carried out at intervals of two to three hours and detailed studies are reported on 3 of the 4 patients on this regimen. Daytime urines were totalled from 8 a.m. to 8 p.m. and night urines from 8 p.m. to 8 a.m.

Repeated urine volume studies over nine- to sixteen-day periods in these 3 patients revealed an average daytime urine volume of 662 cc. compared with night volumes of 1936 cc. Three normal subjects who underwent the same regimen for one day showed a reversal of these results with 1670 cc. average daytime volumes and 758 cc. night volumes. Similar studies were carried out on 5 patients, using the Mosenthal regimen originally designed for renal function studies (H. O. Mosenthal, *Arch. Int. Med.* **16,** *733 (1915)*), and the results were compared with the normals reported by Mosenthal. In these studies also, the sprue subjects revealed a markedly increased nocturnal and decreased diurnal urine excretion.

Postural studies to determine the possible influence of glomerular filtration on the nocturnal diuresis in these patients revealed no changes in blood pressure when erect or recumbent, and several patients who were kept in bed all day as well as at night revealed no alteration in urine volume curves as a result of this procedure. Creatinine chromogen clearance tests on one patient also indicated no change in glomerular filtration. Renal function studies consisting of urinalyses in all patients and specific gravity determinations or urea clearance in 4 patients were carried out, again with negative results.

Because of the known absorption defect occurring in sprue patients, various attempts were then made to investigate the possible relationship between food in the gastrointestinal tract and the delayed diuresis. One patient receiving the constant food and water regimen was studied by altering the time of food or fluid ingestion. Delaying the food intake by postponing each meal four hours beyond the usual time delayed the curve of diuresis by four hours. Likewise, omitting the evening meal and 8 p.m. feeding advanced the curve of diuresis by approximately four hours. Varying the time of fluid ingestion while administering food at the regular intervals, however, had no significant effect on the time of the diuresis regardless of whether two-thirds of the fluid was ingested before 10 a.m. or one-half of it was drunk after 5 p.m. As a result of these findings the authors then hypothesized that water excretion was delayed by the retention of large amounts of fluid in the gastrointestinal tract during the prolonged period of assimilation of food from the malfunctioning bowel. This hypothesis was further

tested by the administration of intravenous fluid. When the patient was given a liter of 5 per cent glucose in water intravenously in the fasting state an immediate diuresis took place with diminution in the subsequent night urine volume. If the fluid was administered after breakfast, however, there was no immediate diuresis and the urine volumes assumed the usual nocturnal curve. Repetition of these water tolerance studies using oral water revealed essentially the same results, indicating the probability that the presence of food attracted or retained fluid in the gastrointestinal tract until the food was absorbed. Moreover, no defect in water absorption was apparent from these experiments.

Additional studies carried out upon a second patient who fasted but drank fluid at the regular times demonstrated that fasting prevented the nocturnal rise in urine volume which regularly occurred when the patient was eating. With this demonstration of the effect of fasting and the evidence noted above the authors reasoned that there might be an optimal concentration of fluid required for absorption of food from the bowel. This would limit the amount of fluid which would be diverted to the intestinal tract and retained there until the time of nocturnal diuresis. The night urine would therefore have a maximum which could not be exceeded regardless of an increase in the fluid ingested during the day. The validity of this prediction was tested by analyzing the effect of several levels of fluid intakes during the day in 2 patients. It was found that with a fluid intake between 2000 and 4000 cc. per day the nocturnal volume increased in both patients, but when daily fluid intake was above 4000 cc. the daytime urine volume increased markedly, whereas the night volume was but slightly enhanced.

It was found that when the daily intake of fluid was low, the nocturnal volume was low, a finding seemingly at odds with the above hypothesis which suggests that the demands of the bowel for fluid would cause retention of constant amounts of fluid and thus maintain the nocturnal diuresis at a relatively constant figure. The authors explain the lowered nocturnal diuresis during low intake days on the grounds that relative dehydration of the body tissues occurs during the day on low fluid intakes and that this is reversed when the fluid is released from the bowel as food is absorbed. In this regard, one patient is cited who complained of afternoon thirst during fluid restriction days, but had no thirst during or following the nocturnal diuresis.

Although these studies appear to confirm the authors' hypothesis, they recognize that only a small number of patients were studied and suggest that other factors may prevent the occurrence of such clear-cut nocturnal diuresis in all sprue patients. Variation in the severity of the absorption defect is suggested as one factor leading to variation in the degree of this urine volume phenomenon, and the presence of severe diarrhea is mentioned as another. The latter, if present, would, by virtue of increasing fecal water loss, reduce the usual amount of fluid returned from the bowel for the night diuresis.

This investigation is worthy of note not only for the unique series of experiments conducted but also for the fact that it reemphasizes the role of food in the gastrointestinal tract in influencing the function of other systems of the body. Although this may be a phenomenon restricted to nontropical sprue and to a specific type of absorption defect, other types of deficiency disease appear worthy of similar investigation. It is unfortunate that no studies are reported on patients whose disease was corrected by specific therapy. In view of the fat absorption defect found in patients with liver disease it would be of interest to determine whether the finding of delayed water diuresis in these patients is also at least in part a manifestation of gastrointestinal dysfunction (*Nutrition Reviews* **9,** *187 (1951)*).

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Letter to the Editor

Dear Sir:

Your review in the November number entitled "Heat Damage to Edible Oils" (*Nutrition Reviews* **9,** *326 (1951)*) may be somewhat misleading unless one clearly understands that the conditions employed in the experiments cited are practically never duplicated by the housewife. There is no reason to question the excellent work of E. W. Crampton, F. A. Farmer, and F. M. Berryhill on herring oil (*J. Nutrition* **43,** *431 (1951)*) on which the article is based; their work confirms earlier results of S. Lassen, E. K. Bacon, and H. J. Dunn (*Arch. Biochem.* **23,** *1 (1949)*), who reported a lowered digestibility of sardine oil after it was subjected to high temperatures.

In the tests of Crampton *et al.* the fat was subjected to a temperature of 275°C. Such edible products as "margarine, salad oils and cooking oils" are never normally subjected to such abuse. The usual temperature for deep fat frying is 205 to 210°C. One certainly does not heat the fats above the smoke point, which in the case of frying oils is about 225°C.

Moreover, neither whole margarine nor whole butter is used for deep fat frying since the charring of the milk solids creates a most undesirable medium for such cooking. In experiments which we have conducted on margarine fat (H. J. Deuel *et al.*, *Food Res.* **16,** *258 (1951)*) in which the fat was continuously heated for frying potato chips over an eight-hour period at 205 to 210°C., we could demonstrate no deterioration in the fat insofar as could be determined from growth rate of young rats receiving 11.5 per cent of this fat in the diet over a twelve-week period. Moreover, potatoes fried in such fat which composed 23 per cent of the diet were likewise well utilized for growth. One should therefore be cautious in postulating deleterious properties to the common food fats since they are not ordinarily subjected to the rigorous treatment required to effect a polymerization.

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On page 55, second paragraph of the article, the statement is made, " . . . that liver cancer could be produced in a shorter period of time by feeding para-dimethylaminoazobenzene, butter-yellow, a pigment used widely to color oils, oleomargarine and other vegetable fat substitutes."

Actually, the colors used in butter, as well as oleomargarine, are primarily the certified dyes, Yellow AB and Yellow OB. The use of para-dimethylaminoazobenzene has been prohibited in the United States in recent years and I believe most dairy companies have not used it since 1900.

A. P. STEWART
Director of Research
Golden State Company, Ltd.
425 Battery Street
San Francisco 11, California

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Dear Sir:

Following personal experience and observations of practices of several other families, it became apparent that the present procedure of adding fluoride ions to municipal water supplies in order to reduce dental caries may be somewhat inadequate to supply the optimal quantity of fluoride needed at the critical times for good tooth development. In many cases the predomi-

tested by the administration of intravenous fluid. When the patient was given a liter of 5 per cent glucose in water intravenously in the fasting state an immediate diuresis took place with diminution in the subsequent night urine volume. If the fluid was administered after breakfast, however, there was no immediate diuresis and the urine volumes assumed the usual nocturnal curve. Repetition of these water tolerance studies using oral water revealed essentially the same results, indicating the probability that the presence of food attracted or retained fluid in the gastrointestinal tract until the food was absorbed. Moreover, no defect in water absorption was apparent from these experiments.

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nant fluid intake of pregnant women consists of milk. During the first several months after birth it is admitted that an appreciable amount of water may be used in the infant's formula, but following that period the youngster's fluid intake consists mainly of whole milk. It appears that the fetal period and the period between 2 and 6 years of age are most important for the ingestion of fluoride to be of use in the sound formation of the first and second sets of teeth respectively. If fluoridation of tap water alone was relied on in the above cases to supply the necessary amount of fluoride, the individuals concerned would possibly not have obtained a sufficient quantity. It must be remembered that it is the amount of fluoride consumed at essential times that is important and not the level in the tap water.

It would also seem logical that better nutritional results would be obtained if the calcium and phosphorus were available to the tissues at the same time as the fluorine.

This premise led me to the practice of adding fluoride (1.5 parts per million) to my family's supply of whole milk, and to our baby formulas. This has been continued for over five years during two pregnancies with the formation of one perfect set of first teeth and the start of another with similar results.

As the infant teeth become available they will be assayed for fluorine, calcium, and phosphorus for comparison with the results presented in "Fluoride Content of Teeth," *Nutrition Reviews* **10,** *28 (1952)*.

It appears that the fluoridation of whole milk should be seriously considered by nutritional authorities to insure adequate fluoride intake. This should in no way detract from the similar treatment of the water supply, for these two sources of fluids only complement each other. Condensed milk should, of course, not be prepared from fluoridated whole milk.

Amos E. Light
49 Colonial Parkway North
Tuckahoe 7, New York

(*Note:* Dr. Light's letter is published because of the suggestion that fluorides might be more efficacious in preventing dental caries if they were present along with calcium and phosphorus during tooth development. However, since fluorine is a highly toxic element when excess amounts are consumed, it should not be added to food materials except under very carefully controlled conditions; every precaution must be taken to guarantee that individuals do not receive excessive amounts of fluorides due to the simultaneous consumption of more than one food material fortified with a fluorine-bearing compound.—*Ed.*)

Letter to the Editor

Dear Sir:

Your review on "Neovitamin A" which appeared in the October 1951 issue of *Nutrition Reviews* (**9,** *315 (1951)*) was of great interest to us. We would like to call your attention to a typographic error in the paragraph discussing the paper by P. L. Harris, S. R. Ames, and J. H. Brinkman, *J. Am. Chem. Soc.* **73,** *1252 (1951)*. In the concluding sentence of this discussion, mention is made of "an equilibrium mixture containing neovitamin A and all-trans vitamin A in the ratio of 1:1 to 2:1 . . ." The ratio mentioned in the original paper, page 1253, is 1:10 to 2:10.

Stanley R. Ames
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Distillation Products Industries
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